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THE SMALLEST RESONANCE ENERGY TRANSFER ACCEPTOR FOR TRYPTOPHAN

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Diazirine compounds have found widespread use as photoactivatable reagents. In this work we report on the extension of the utility of diazirine ligands in protein science. The diazirine moiety, which absorb between 330-380 nm, is used as an acceptor for tryptophan (Trp) and Trp analogs, like 5-fluoroTrp, in resonance energy transfer (RET) experiments. Distances up to 18 Å can be determined with high accuracy. The principle is demonstrated for a diazirine derivative of mannitol, 2-azi-2-deoxy-D-arabino-hexitol (azi-mannitol) and a dozen single-Trp-containing mutants of the membrane bound mannitol transporter, EII^{mtl}, from *E. coli*. The diazirine moiety is photostable under the experimental conditions. The ambiguity about the exact position of the chromophoric group relative to the ligand due to the use of a flexible linker is absent in azi-mannitol, since the chromophoric group is an integral part of the substrate. The small size of the diazirine group allows its isosteric introduction in biological ligands without a large effect on the biological activity. In conclusion, the versatility of Trp as an intrinsic spectroscopic probe of proteins and the small size of the diazirine group make this a very attractive donor-acceptor couple for accurate RET distance information in protein chemistry.